

REVIEW ON HONEY BEE COLONY COLLAPSE: PROBLEMS, CONSEQUENCE AND
SOLUTIONS

THOMAS F. FREEMAN HONORS COLLEGE

SENIOR THESIS

BY

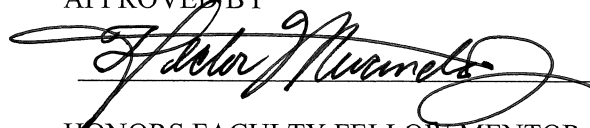
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ABSTRACT

This thesis manuscript is a review on how climate change, nutritional stress, and other factors contribute to colony collapse disorder (CCD). This is a phenomenon when majority of adult bees vacate their hive to die, leaving what little what little is left of the colony to fend for themselves. This phenomenon is considered a serious threat to global food biosecurity. The big question is on when and why honeybees abandon the colony and die. This phenomenon remains a mystery. Unable to study their carcasses for analysis to better serve the pollinator species, there has been a rise in research focused on investigating the key drivers to CCD. My research suggested that the problem of CCD is complex and tied up to the greater problem of climate change and human activities. The focus of this paper is to understand how climate change in a rapidly warming world has contributed to the demise of honeybees. I focused on isolating the major factors and reviewed the data on global warming and its specific impact on honeybee colonies, where many other drivers participate. Evaluation of the interactive and synergistic effects of key drivers revealed how they affected mutually beneficial relationships, and ultimately the ecology of the bees and their habitat. I also investigated and assessed the overall interactive effects of potential synergies between climatic and nutritional stresses and understand how they affect honeybee colonies feeding responses. Notions were evaluated based on colony growth, composition of brood, mortality, total pollen and syrup collection. My review highly suggest that sociality, honeybees' interdependence on one another are crucial in buffering the negative effects of environmental perturbations and increasing their resilience.

HONEY BEE COLONY COLLAPSE: PROBLEMS, CONSEQUENCES AND SOLUTIONS

By

MARIELA RODRIGUEZ

Texas Southern University, 2020

Dr. Hector C. Miranda, Jr., Advisor

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VITA

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DEDICATION

I dedicate this piece to all future generations of naturalists in hopes of promoting the value of biodiversity.

ACKNOWLEDGEMENT

I would like to thank the Thomas F Freeman Honors College for allowing me the opportunity to inflict knowledge upon the importance of conserving and protecting wildlife. It is my hope to inform others on collateral damage done within our ecosystems as honeybees continue to be targeted. I would like to thank Dr. Hector Miranda for his tireless dedication in helping me throughout my academic studies, giving me perception in relation to my research and active participation. Without his guidance and endless inspiration my spark of interest in biodiversity conservation wouldn't have been realized in the conservation of endangered species. I am indebted to my mentor and former softball coach Rae Gaut, thank you for seeing potential in me, as well as your friendship, tough love, and great sense of humor. A special thank you to Dr. Ayodotun Sodipe for believing in me and pushing me to my limits to become a sharper scholar. I would like to extend my gratitude to my close friends and family who have shown me endless support and who continue to stimulate discussions about my research.

CHAPTER I

INTRODUCTION

Background Information

After hitting the mainstream platform of media, bees have finally hit the spotlight and the public sees how urgent it is to act now. It is my understanding that there is a gap between awareness and understanding. Many analyses have been published and caught the attention of the public eye about how bee populations are declining and at risk if no action is taken. Efforts are to be made to conserve their existence but for these conservation efforts to be passed they require public support and participation. A survey was conducted to measure public understanding of bee diversity and found that although 99% of respondents believed that bees are critical or important, only 14% were able to guess within 1000 that actual number of species in the U.S.. Their findings show that even as scientific research on bees have rapidly expanded, the public remains largely uninformed on the subject, especially with regard to the wealth of bee diversity in the U.S (Wilson et al, 2017). Such lack of knowledge could therefore lead, although of good intentions, to irrelevant action that is misguided, or even counterproductive for the protection of threatened pollinator species (Wilson et al, 2017). Bees around the world are being decimated; dying off in vast quantities. Oklahoma lost 85% of their bee hive in 2016-2017 annual loss, based on of bee report. In 2018-2019, the survey reported loss of 40.7% representing a slight increase over the annual average of 38.7%. Nonetheless, winter losses of 37.7%, were the highest winter loss reported since the survey began 13 years ago, and 8.9 percentage points higher than the survey average (University of Maryland report , 2019)

The almond harvest in California is the biggest market for beekeepers; they send majority of their bees to the almond orchards; A single bee pollinating 1000 flowers a day being one of the most bee-dependent crops in the world. About 35% of our food is directly dependent upon bee pollination, the remaining 65% is much indirectly dependent but hay, alfalfa and clover for cows, all of our dairy is dependent on bee pollination. All our berries and nuts, coffee, cannabis, and other non-dependent plants benefit from “buzz pollination” where pollen held less firmly by their anthers can then spread better through the air. It is now thought by the many entomologist we could have full colony collapse across the world within 10 years; the cost being massive to society, prices of food will raise, and poverty will increase. Thus, CCD is much more serious than most people realize because all the wild bees have now been infected. Wild bees represents 80% of where pollination comes from, and remaining 20% comes from managed honeybees.

Review of Literature

Systematics and Evolution of Honey Bees

The honey bees (genus *Apis*) are the most recognized of all insects, primarily because of their role in pollination and agriculture. All bees belong to the superfamily Apoidea. There is an estimate of about 20,000 species in about 700 genera distributed in 10 families (Malyshiev 1968, Michener 1969). Most of the genus *Apis* are distributed across Asia (Arias and Sheppard 2005). The western honey bee, *Apis mellifera*, was historically distributed throughout sub-Saharan Africa, Europe, parts of western Asia, and the Middle East. It is the only species of honey bee that has undergone substantial domestication to which all commercial honey bee descended. This species is thought to have split from its close relative, *Apis cerana*, between 6 and 25 million years ago, and expanded westward to colonize parts of Asia, Europe, and Africa

(Sheppard and Meixner 2003; Ramirez et al. 2010). Different lineages of *A. mellifera* were subsequently transported to different parts of the globe during the European colonization (Sheppard 1989).

Phylogenetic analyses based on both mitochondrial and nuclear markers have shown that there are three distinct *Apis* groups: cavity nesting bees which include *A. mellifera*, *A. cerana*, *A. koschevnikovi*, *A. nulensis*), giant bees (*A. dorsata*, *A. laboriosa*, *A. binghami*, *A. nigrocincta*), and dwarf bees (*A. florea*, *A. andreniformis*) (Arias and Sheppard 2005; Raffiudin and Crozier 2007) (Fig. 1A).

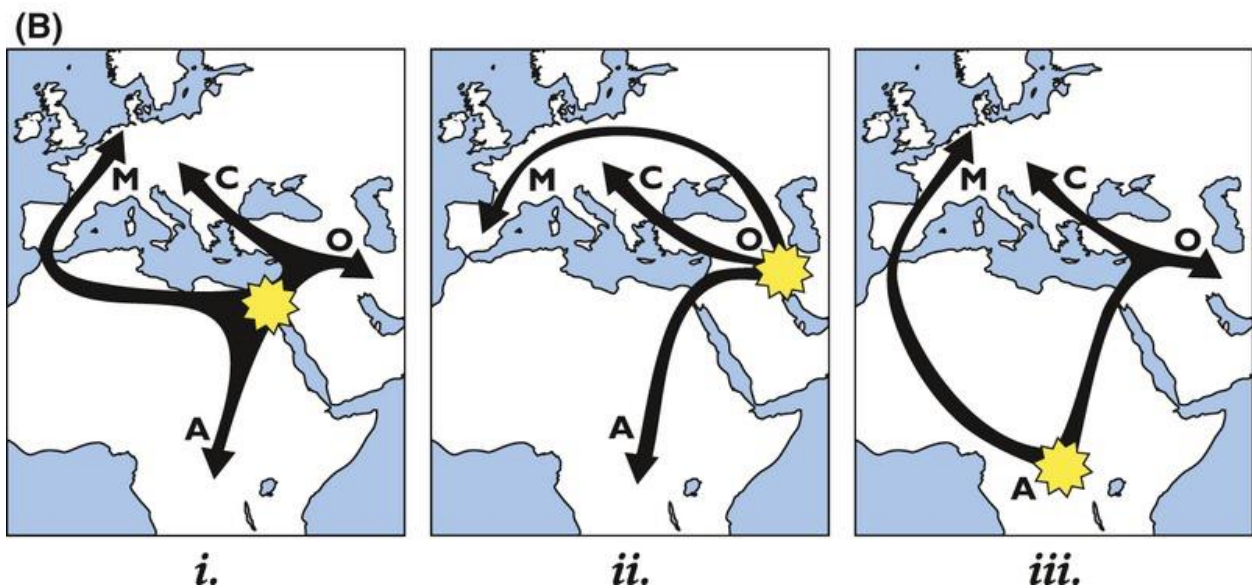
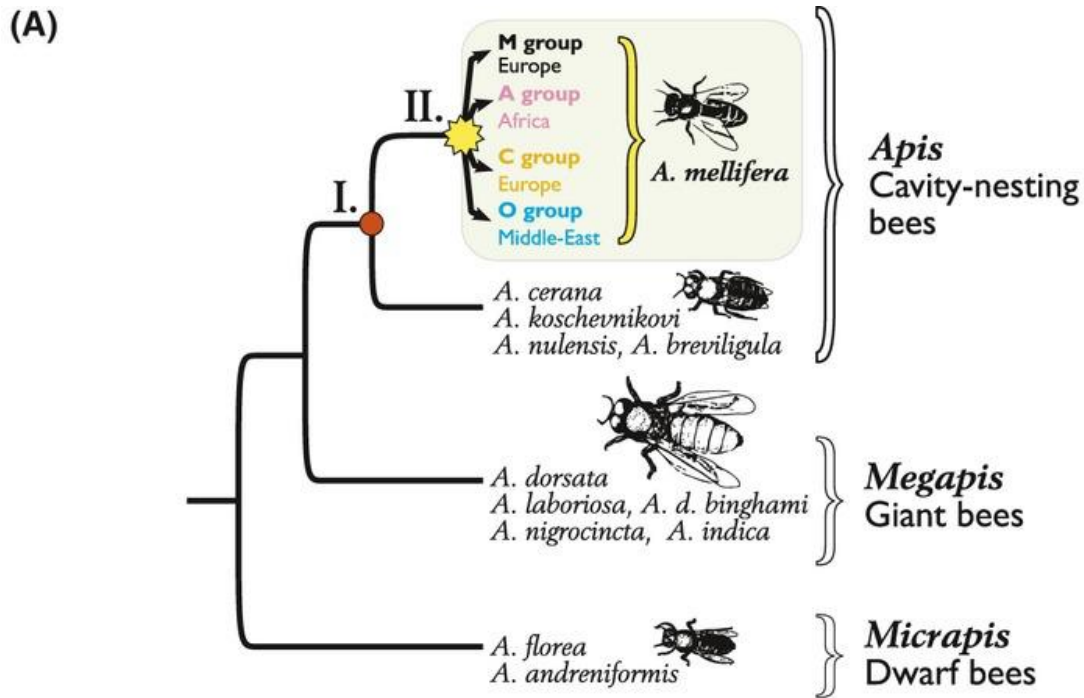


Figure 1. Evolution of *Apis mellifera*. (A) Phylogeny representing the three clades of *Apis*. All of the 10 extant(living) *Apis* species, apart from *A. mellifera* are found only in Asia. Node I represents the split between *A. mellifera* and other cavity-nesting bees. Node II represents the most recent common ancestor of extant subspecies of *A. mellifera*. (B) Three hypotheses that have been proposed for the origin of *A. mellifera*. (i) An expansion from the Middle East,

involving colonization of Europe via two routes, one eastern and one western (ii) .An expansion from the Middle East, which did not involve the western colonization route into Europe was suggested on the basis of trees constructed from mtDNA (Garnery et al. 1992). (iii) An origin in Africa was proposed by Wilson (1971) and an expansion out of Africa via both an eastern and western route was suggested by the analysis of >1000 SNPs by Whitfield et al. (2006). The yellow star corresponds to node II in the upper panel. (Figure adapted from Han et al. 2012).

Bee Biology and the Social Structure of Bee Colonies

Honeybees are very social insects that rely on one another in well-organized family groups. A bee colony consists of three kinds of adult bees: workers, drones, and a queen. The social structure of the colony is preserved by the presence of the queen and workers and depends on an effective system of communication. The allocation of chemical pheromones among members and communicative “dances” are responsible for influencing the activities necessary for colony survival. Labor activities among worker bees depend primarily on the age of the bee but will deviate from this depending on the needs of the colony. Breeding and colony strength depend on the queen but will vary with the quantity of food; pollen and nectar, and the size of the worker force. As the size of the colony increases up to a maximum of ~60,000 workers, so does the efficiency of the colony.

Queen bees

The queen has another important quality she adds to the colony other than reproduction. She produces many pheromones from her mandibular glands one called “queen substance” that serves as a social buffer unifying and helping to give individual identity to a bee colony. When it

time to mate the queen will fly away putting as much distance between herself and the hive. This serves the purpose of avoiding inbreeding and maintain genetic diversity. Her pheromones allow drones to locate her for fornication, after returning to the hive she begins laying her fertilized eggs in about 48 hours. The quality, size and disposition of the colony heavily relies on the queen's genetic makeup and along with the drones she mated with. Once she's returned to the hive, she releases several sperm from the spermatheca each time she lays an egg designed to become a worker or queen. If she lays a larger eggs characteristic for drones, then she will not fertilize it. If the queen was unable to leave the hive to mate, she will have no choice but to lay an unfertilized eggs thus producing only drones (male bee) this risks the health of the colony. Depending on how much food she receives and her worker bee force capability to prepare an adequate amount of beeswax cells correlates to how many viable eggs she produces. After 3 days the larva starts to hatch.

Once the queen has fulfilled her duties and can no longer produce viable queen substance she is dethroned, or rather the daughter queen supersedes her. She can be dethroned for a number of reasons; she's a low functioning queen, out of eggs, or she was not breeding well. Sometimes the queen thinks she's still doing a good job, so in this case she won't swarm, but will be replaced. (Offord, 2016)

These supersedure queens are raised in worker cells modified to hang vertically in the middle of the comb surface (supersedure cell). This is specifically done to hide the emerging queen from the queen the bees wish to replace. Queens made through supersession are of better quality, since they are fed bigger portions of "royal jelly" during development. When the supersedure queen emerges there's usually a battle between them for the throne. The queen bees

is the only bee that can sting and survive. Once stung by the stingers they will die, and the survivor takes her place.

New queens arise in certain circumstances, emergency, supersedure, or swarming. When an old queen is unintentionally killed, lost, or removed, the bees select younger worker larvae to produce emergency queens. A potential queen that is genetically similar to a worker but is fed a rich diet the 1st couple days of development that trigger a host of changes in gene expression This, then dramatically alters the morphology, behavior, and longevity of the queen (Evans, 2020)

Queen cells are a special type of cells that's is produced in the hive and looks like a little teacup, where it is located will help identify the potential outcome (Maigatter, 2016). A teacup with an eggs or larva is now considered a queen cell. Queen cells produced in preparation for swarming are found along the bottom margins of the frames or in gaps in the beeswax combs within the brood area. When the colony is in abundance and is healthy enough to reproduce it will produce a swarm cell filled with royal jelly to split to produce more superorganisms at additional nesting sites. This is when swarming comes into play. The old queen is usually prepared for this is her second year as queen on the hive. The worker bees will place her on a diet so she may fly with ease. With her she will take worker bees, foragers, and other bees to make sure her new hive can operate at maximum proficiency, they gorge themselves on honey before taking off for the strenuous mission ahead. She will leave the bits of the colony behind with the virgin queen. Swarming is a positive sign that the colony is healthy and the former queen has done her job, putting all the preparation in place for the hives success.

Drones

Drones are male bees that solely serve to fertilize the queen once they are sexually mature then and die promptly after mating. Although they don't serve in any other way they are considered to be vital to maintaining genetic diversity, and thus to colony health. They are the largest bee in the colony and eat up to three times more than any other bee, the worker bees are the ones that supply the food. There is a delicate balance to how many drones should be left to live, if there are too many the colony should take on additional stress on the colony's food supply. When the seasons shift the bees start getting ready for the scarcity they'll be subjected to since pollen/nectar won't be as prominent to come by, the drones will be forced out into the cold and left to starve. It's never been recorded before that any drone goes out and collect pollen for the hive, since the only skill they have is mating. Drones cannot provide the hive any other form of aid to be welcomed to stay. The only way the hive will allow them to stay indefinitely is if the hive loses their queen.

Worker bees

Worker bees are sexually undeveloped females with specialized structures like brood food glands, scent glands, wax glands, and pollen baskets, which allow them to perform all the labors of the hive. Underdevelopment of the workers ovaries is believed to be due to the pheromones released by the queen and presence of brood they need to be attended to. They are in charge of all the day-to-day operations that allow the hive to operate at such high capacities. Performing tasks like cleaning and polishing the cells while removing debris, nursing the brood, caring for the queen making sure she is constantly fed with the royal jelly, handle incoming nectar, building beeswax combs, guard the entrance, and air-condition and ventilate the hive during their initial

few weeks as adults. Later as field bees they forage for nectar, pollen, water, and propolis (plant sap). The life span of the worker during summer is typically 6 weeks. Workers reared in the fall may live as long as 6 months, allowing the colony to survive the winter and assisting in the rearing of new generations in the spring before they die (MAAREC, 2014).

The only way a worker bee will develop their ovaries is if they lose their queen, this is deemed an emergency situation, so they adapt to the situation change their behavioral tendencies and start laying some unfertilized eggs so they may have a chance at survival. The only way they do this is if they've been queen-less for a week+. However, laying workers (Figure 5) may be normalized in "queenright" colonies during swarming season and when the colony is headed by a poor queen.

The larvae are responsible for producing 2 types of pheromones, the chemical odors release serve as communication to the beehive. They signal workers for colony tasks, especially concerning larval care (Burlew, 2013). For example, brood ester pheromone (BEP) increases the production protein in workers that inhibits their ovaries and regulates the capping of brood cells. Another brood pheromones called E- β -ocimene, regulates the activities of workers, managing the nurse-to-forager ratio. (Burlew, 2013) . thus, when a hive loses their queen, brood production ceases and associated pheromones deplete, the hive will try and counter the negative effects, hundreds becoming fertile laying workers.

The only problem with this, since laying workers never go out and fertilize their eggs like the queen did, they only lay unfertilized eggs. Unfertilized eggs result in drones while fertilized eggs result in workers or queens. This becomes problematic for the hive since a drone's only function is mating with another queen. So while this preserves and disperses their genetics onto other hives, their colony will suffer as day operations diminish and food remain unstocked. If

there is no intervention, the hive will die off. While this is true when a hive becomes overran with laying workers, you can't requeen a laying worker. They will not accept a new queen easily because they consider themselves a queenright colony, they will try and kill the new queen (Deeley, 2014). Laying workers will try and make their own queen, so they lay multiple eggs in a queen cell in a desperate attempt to raise their own queen (Deeley, 2014) (Figure 3). Colonies with laying workers are usually easy to spot; they usually stack 5-15 eggs/cell. This alone isn't enough to characterize a laying worker hive because new queens sometimes do this as they are overwhelmed with energetic desire to start fulfilling their duties, but they will quickly correct this. A queen will start laying their eggs starting from the center of the cell, while laying workers tend to start from the margins of the cells and the eggs are laid on pollen (Figure 4). You may also see spotty drone brood in worker comb (although this may also be a sign of a failing queen). Some of these eggs laid do not hatch, and many of the drone larvae that do hatch do not survive to maturity in the smaller cells (Deeley, 2014). A strategy an apiarist tries to mitigate the negative effects surrounding a queen right colony is to introduce open brood pheromones once a week for 3 weeks, or until they start to raise their own queen. This pheromone is basically the same pheromone given off by an uncapped brood. It is used to suppress worker ovaries in a colony that has become queenless. The time frame is essential for this method to work, and it allows the laying bees enough time to adjust, which allows them to either raise their own queen, or accept the introduction of one as a larvae.



Figure 2. A desperate attempt of laying workers to lay their own queen.

<https://www.beverlybees.com/laying-workers/>



Figure 3; multiple eggs randomly placed over hive is a sign of laying workers.

(<https://www.beverlybees.com/laying-workers/>)



Figure 4. Another sign of laying workers are eggs being laid on pollen.

<https://www.beverlybees.com/laying-workers/>

The Role of Honey Bees as Pollinators in Natural Systems

Pollinators are in decline around the world, and these declines are best modeled in honeybees. There are combinations of stressors. Both biotic and abiotic stressors are of great significance to colony health. Bees are a main source of pollinators that contribute to a variety of plant species, without these key pollinators that established their mutualistic relationships with flowering plants millions of years ago, we won't have a sufficient replacement to keep up with the maintenance of a stable ecosystems that biological communities require. Pollination is one of the most important ecological interactions involved in maintaining biodiversity because flowers serve as a means of nutritional value to bees (Martins, 2013).

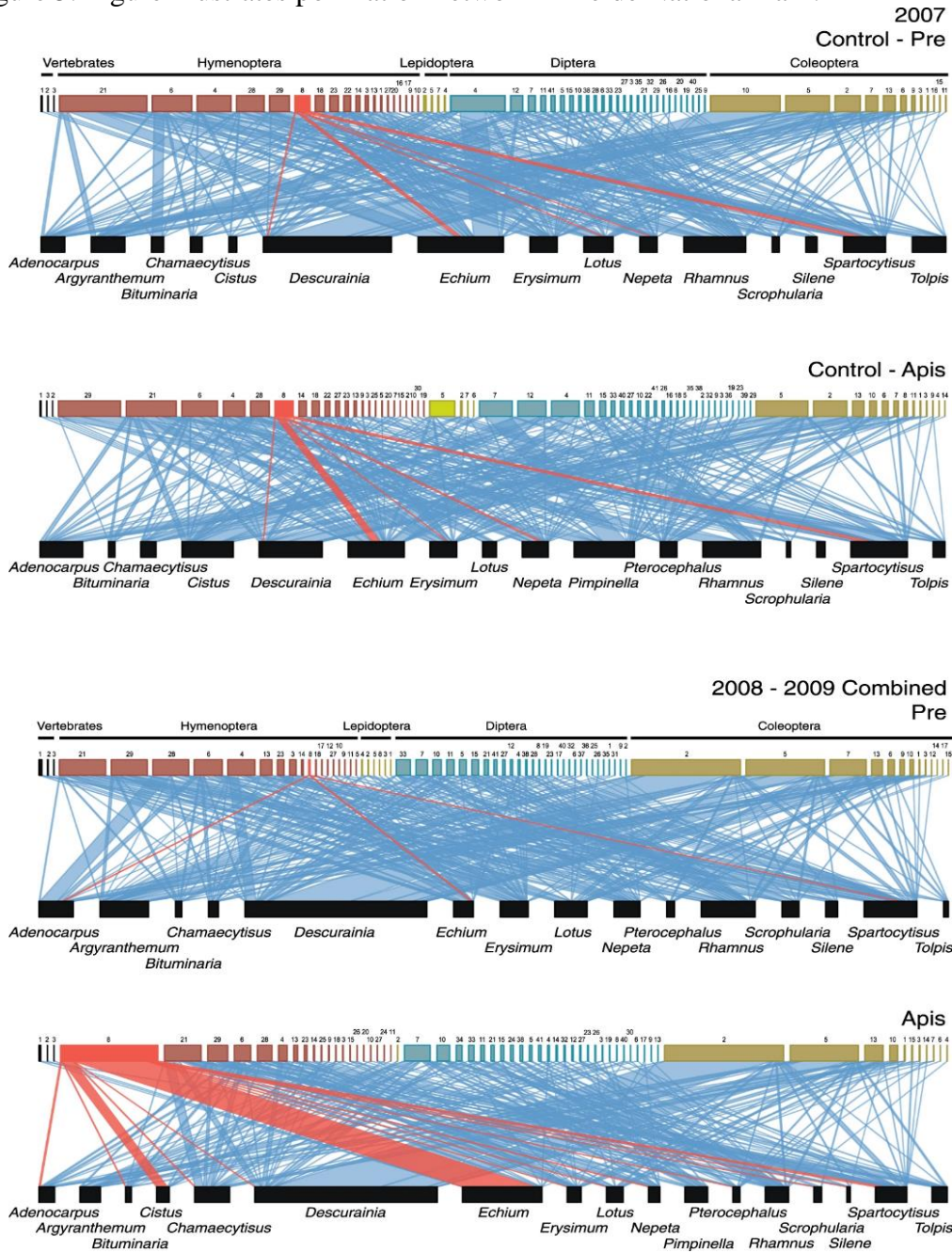
The *Apis mellifera* is considered to be a generalist insect catering to a variety of both generalist and specialist plant species. Thus they have been physically introduced repeatedly to many areas around the globe through time (Aslan et al, 2016) in efforts to counteract the implications left by native bees that succumbed to non-sustainable farming practices, illness or habitat loss. The introduced honey bees appear to have replaced pollination services formerly provided by diverse groups of wild type bees. This is a perfect example of an acute solution whose purpose should be to buy some time for other species to recover. It shouldn't derail efforts to protect the 4,000 species of native bees in North America. The pollination efficiency is increased 5-fold when wild bees are interacting in conjunction with the introduced honeybees. (Greenleaf and Kremen, 2006). According to Greenleaf and Kremen (2006), "we need a solution that will remedy the actual problem long term because the problem is, if we don't protect the wild pollinators, we don't have a backup plan."

One of the factors that push us to focus on the wealth of biodiversity is the fact that *Apis mellifera*, although physiologically able to cater to a wide range of native plants once naturalized, can prove itself to be counterproductive in nonnative natural areas. Introduction and establishment of nonnative *A. mellifera* as a pollinator and forager carries the potential to alter mutualistic and competitive interaction networks (Aslan et al 2016). The introduced *Apis mellifera* may directly enhance native plant fitness via pollination, or indirectly reduce native plant health by pollinating nonnative invasive species. This cross pollination can alter ecological relationships that arose over evolutionary timescales (Traveset and Richardson, 2006). We need to protect the patterns of biodiversity and try to maintain and restore ecological connectivity. (Schmitz, 2015). A three-year (2007-2009) field experiment in Teide National Park (Tenerife, Canary Islands) was conducted to see the ecological influence of beekeeping and *Apis mellifera*

have on biodiversity. The study was done in a natural ecosystem and compared the plant reproduction success before and after the establishment of beehives were integrated in the pollination network structure. The study correlated reduced efficiency with beekeeping, providing insight on how exactly the interaction links in the pollination networks were disrupted by *Apis mellifera*. Data also supported reduced biodiversity due to the frequency of visitation of the honeybee diminishing pollination services by wild pollinators (Figure 5). High-density beekeeping in natural areas appears to have lasting, more serious negative impacts on biodiversity than was previously assumed. Around 2,700 beehives were introduced in the national park at the peak of spring bloom. While comparing pre and post-establishment stages of beehives of honeybees on the pollination network structure, we also pay close attention to the consequences on plant reproductive success by using two complementary field experiments (comparing the reproductive outcome at individual level in five plant species under presence/absence of honeybees, and by using distance from apiaries as a proxy of the relative abundance of honeybees in one plant species; honeybees, and by using distance from apiaries as a proxy of the relative abundance of honeybees in one plant species; see below (Valido, 2019).

Figure 5 illustrates how pollination network in Teide National Park. 2007 is used as the control year because no significant changes were made since *Apis mellifera* was practically absent for the 1st season, thus can be termed as the pre-establishment stage of beehive on the pollination network structure. 2008-2009 were combined so we can interpret the raw data collectively so we can observe the change in pollination network. Size of boxes is proportional to the number of visits recorded per species. Link width represents the frequency of observed plant-pollinator interactions. *A. mellifera* and its interactions are in red.

Figure 5. Figure illustrates pollination network in Teide National Park.



The onset of the beekeeping period (2008-2009) triggered considerable shifts between the *pre*- and *apis*-periods, leading to a reduction in the number of pollinator species but also in

interaction links (Valido, 2019). Since honeybees tend to monopolize a considerable portion of interactions, decrease in wild pollinator interaction due to exploitative competition of nectar increasingly depleted since the introduction of *A. mellifera* can be seen. Competition has caused a reduction in number of pollinator species and interaction links shifting the network parameters thus contributing to a significantly lower connectance (C) under beekeeping activity ($P < 0.05$) especially expressed in 2008-2009 than the control year ($P < 0.001$). However, beekeeping did not alter interaction diversity, linkage density or the interaction strength asymmetry ($P > 0.05$, Supplementary Fig. 5).

CHAPTER II

ECONOMIC IMPACT OF BEEKEEPING IN AGRICULTURE

The Role of Honey Bees in Agriculture

The Western honey bee, *Apis mellifera*, is perhaps the most important insect pollinator of agricultural crops worldwide thus making services surrounding pollination the most economically valuable ecosystem service. Numerous food commodities such as almond and apple, rely on honey bees for fruit, and vegetable, and other resources (Klein et al. 2007). This dependence of agriculture on honeybees has steadily increased during the last decade (Aizen et al. 2009). In the US, the value of honey bee pollination services, including bee-derived products such as honey, wax, and pollen, is believed to be between 10 and 14 billion dollars annually (Calderone 2012). In addition, a variety of honey trade as international commodities (vanEngelsdorp and Meixner 2010).

The potential collateral damage we face as honeybees continue to disappear are detrimental, as we would face economic and ecological impacts. Bees play a crucial role in many ecosystems across the globe, including agriculture with pollination adding over \$15 billion in crop value every year in the US for crops like nuts, vegetables, and fruits according to the US department of Agriculture. CCD also affect the beef and dairy industries as they pollinate clover, hay and other forage crops. As they die off due to lack of pollination this directly impacts the cost of feeding our livestock. Thus, increasing beef and milk prices at a grocery store. This is an example of how important bees have direct influence in not only our food supply but as well has adverse contributions to the demand (Rucker et al 2016). This disorder will lead to increased imports of produce from foreign countries raising the U.S trade deficit (Rucker 2016).

A study by the Royal Society highlights what exactly is at stake if we do not improve pollinator diversity (Klein et al., 2006). In Brazil "Passion fruits...are hand-pollinated through expensive day-laborers as the natural pollinators, carpenter bees, are hardly available because of high insecticide use in the agricultural fields and the destruction of the natural habitats," said lead author Klein. As you can imagine, the biodiversity of flowers and plants are highly dependent upon pollinators so this sways the integrity of the environment. This tedious vegetation method is of great significance to the population. Although it's not as efficient as the natural pollinators, this is the fate we face. We see how this hand-pollination technique could increase the demand of people needed, thus creating jobs-- what we will also see is demand exceeding supply and the price of goods increase. Although fitness is a big part of sociocultural life in Brazil, standing in second place of having the biggest fitness industry, they are already seeing a reflection in obesity rates as prices rise for fruits and vegetables swaying individuals toward less nutritious alternatives. Whereby diets push from consumption of fiber and carbohydrate into saturated fats and sugars (Rtveladze, 2013).

CHAPTER III

CAUSES OF COLONY COLLAPSE DISORDER

The Varroa Mite Infestation

In 1984 the *Varroa* mite was introduced into the United States, it is a parasite that resides on the back of bees and injects virus, in particular deformed wing virus. A bee's lifespan is 30 days, and out of those 30 they leave the hive for 9 days to pollinate. The service they provide to the hive is bringing the pollen. Once they do that they die off. At present, the average time for pollination is only 4 days and this is due to the mites. Colony infestation by the *Varroa destructor* (Anderson and Trueman, 2000) mite reduces colony resource reserves during winter months and slowly feeds off the body fat and blood. A way the bee protects themselves is to groom themselves very aggressively so that they brush off the mites.

In order to fight the mites they've been using toxic insecticides. One insecticide that proved to be counteractive in fighting off mites and actually negatively affects the honeybees were neonic clothianidin. This compound leads to honeybees decreasing the proportion of bees that groomed intensively, and affected genes associated with neurodegenerative processes.

Uncontrolled Use of Pesticide

Taking a closer look at pesticides, I examined how the two most found pesticides on hives; coumaphos and fluvalinate, used to control *Varroa* mites impact on genome wide gene expression patterns of honey bee workers. While acute doses of pesticides can kill individual honeybees and colonies on the spot, chronic exposure at low doses lead to sub-lethal effects in individual bees, which in turn, may result in colony level effects (Schmehl, 2014),

Those compounds specifically affect genes involved in detoxification, behavioral maturation, immunity and nutrition were found to have significant changes. (Schmehl, 2014)

Exposure to pesticides modulate expression of genes involved in immunity and behavioral maturation. For example, exposure of pesticide neonicotinoids lead to reduced activity of the NF- κ B immune signaling pathway and increased concentration of deformed wing virus, suggesting that if honeybees become compromised by pesticide exposure they may be more susceptible to pathogen infection. Pesticides also influence endocrine pathways. A primary hormonal regulator of adult worker behavior is juvenile hormone III (JH), which is synthesized from methyl farnesoate (MF). When concentration of JH rises it drives behavioral maturation transitioning from a nursing brood to a foraging honey bee worker.

Further exploration between the association of nutrition and pesticide-regulated gene expression patterns demonstrated that bees fed a pollen-based diet exhibit reduced sensitivity to a third pesticide, chlorpyrifos (Schmehl, 2014). The *Apis mellifera* genome is characterized by the scarcity of genes associated with detoxification, being able to convert toxic substances into less toxic substances able for excretion. Thus making the honeybee vulnerable to specific pesticides, especially those in combination emerging in real field environments (Gong and Diao, 2017) . After looking to mechanisms involved in detoxification of xenobiotics/pesticides in honey bees, the global transcriptomic and proteomic approach have allowed understanding of how detoxification mechanisms in honey bees involve multiple genes and pathways along with changes in energy metabolism and cellular stress response.

The Cytochrome P450 gene, is highly implicated in the direct detoxification of xenobiotics /insecticides in honey bees and their expression can be regulated by honey/pollen constitutes, resulting in the tolerance of honey bees to other xenobiotics or insecticides. The

expression levels of detoxification genes like P450s was upregulated in response to pollen feeding, suggesting that these pesticides and components in pollen curb similar molecular response pathways. Therefore, it can be deduced that pollen-based diets reduce workers pesticide sensitivity to toxic substances in nature and providing high quality nutrition will in turn improve resistance to pesticides. With the wide use of insecticides in agriculture, understanding the detoxification mechanism of insecticides in honey bees and how honeybees fight with the xenobiotics or insecticides to survive in the changing environment will finally benefit honeybees' management. (Gong and Diao, 2017)

Urbanization

Houston has an occupancy of 89.7% and rising with construction of multi-family housing units on the decline (Freedman, 2019) the need for housing in cities grows annually. Houston is the biggest town among the top 100 of 50,000 residents and the only with a population exceeding 2 million (Freedman, 2019). With disruptions from natural disasters like hurricane Harvey impacting the housing market unable to keep up with, rent seems to be on the rise due to lack of housing. This may then lead to a surge in developing rural areas to meet the needs resulting in more construction. Abiotic factors associated with urbanization affect the ecology by altering natural and historically agricultural landscapes with development of cities, suburbs and supporting infrastructure like the cement roads. Abiotic changes in the urban environment, such as the “urban heat island effect,” have caused shifts in phenology. Urban heat island effect is a phenomenon described as cities being warmer compared to rural communities due to the lack of trees and increased concrete. Its these surfaces that are responsible for trapping heat causing cities to remain warmer during the day and night. Energy from sunlight is absorbed in the

concrete in the form of heat. This heat diffuses back into the system, thus keeping cities warmer in contrast to rural areas. Plants in the cities bathe in the sun all day and still experience warmer nights without discriminating between summer or winter throughout the year. Naturally, warmer temperatures can bring a halt to winter dormancy earlier or allow more heat loving plants to be grown in cooler regions (Wright, 2017). This alters thermal conditions and breeding seasons by indirectly extending urban warming (Miles, 2019). According to the University of Florida, another consequence of concrete surfaces is increased pH in soils. The runoff from cement causes alkaline heavy soils. With the soils integrity compromised this dampens the availability of nutrients and ions for plant roots to absorb (Wright, 2017). This nutrient deficiency causes chlorosis presenting itself as yellowing of the leaves. A good way to counter the alkalinity of the soil is to add magnesium but this just treats the symptoms of a deep-rooted problem.

Other abiotic changes in urban areas, including water availability, pollution, and habitat fragmentation, have resulted in changes to physiology, behavior, and population abundance. According to Martins (2013). While some species can physiologically acclimate or genetically adapt to the abiotic urban environment, many species are expected to decline in abundance. Native species vibrancy tends to decline in urban areas because their surrounding biotic and abiotic environments are unlike any natural environment and are becoming challenging to tolerate by bees and plants. For instance, between 1960s and 1980 there was a significant spike in urbanization thus increasing use of agricultural chemicals in the region these conditions were prescribed to be the main causes of the marked decline in bee abundance (Martins, 2013). To understand how urbanization and climate change we need to see how bees respond to environmental stress. We consider two important physiological responses: oxidative stress and inflammation. These factors are chosen because they exhibit genetic control and plasticity that

allow investigation on how species acclimate, endure and prosper in constantly changing conditions.

Oxidative stress occurs when pro-oxidants like reactive oxygen/nitrogen species (ROS/RNS) exceed the capacity of antioxidant defenses available in species that function to remove and detoxify the excessive levels of ROS/RNS. When left unchecked ROS/RNS reactive molecules react and damage important macromolecules. Oxidative damage to lipids and proteins can induce a conformational change which in turn changes their function, and possibly render them dysfunctional. Oxidative damage can also damage nucleic acids leading to transcriptional errors. Cellular dysfunction and apoptosis occur when the cells are overwhelmed with oxidative stress which is linked to increase susceptibility to many diseases.

Oxygen is present in all aerobic cells which is the starting material needed for activation of ROS/RNS and trigger oxidative attack that function to fight off pathogens. Thus, mild oxidative stress is a default setting and any deviations from this delicate balance would trigger apoptosis and pathology. Thus, the activities that increase cellular respiration and infection are likely to increase internal oxidative stress.

Inflammation is a key first line defense against harmful stimuli and are triggered by innate responses to infection, damage, or harmful substances. Inflammation induces increased vascular permeability and inflammatory mediators to recruit of immune cells via chemotaxis and release ROS/RNS to enhance oxidative stress to neutralize the threat.

Urban areas contain several environmental stressors that are pronounced differences compared to rural environments. They include (i) chemical pollution, (ii) noise pollution, (iii) artificial night light pollution, (iv) infectious disease and (v) diet quality.(Isaksson, 2015) factors like these are known to trigger linked inflammation responses and oxidative stress.

Urbanization provokes serious consequences for the maintenance of bee diversity mainly due to the reduction of resources, including nesting sites and flower (food resource) abundance (Martin, 2013)

Climate Change

Considering the climate, in 2020 illustrated the profound impact of our rapidly warming world. We've seen more frequent flooding in the central US, longer periods of wildfires in California and the Amazons, and droughts across the Great Plains. Increasing greenhouse gas concentrations were always expected to have a significant influence on global climate on a timescale of decades to centuries (Hughes, 2000), but accumulation of data of past climatic variation suggests that the recent climatic and atmospheric trends do not reflect past results that show the linear relationships with species physiology, distribution and phenology. As law of large numbers suggests the climate may even out eventually, we need to start focusing on developing adequate supplies of “workhorse” species: models, locally adapted native plants that are high in abundance, amplitude and easy to propagate for vegetation analysis, undertaking more focused collections in both “bad” years and “bad” sites to maximize the potential ability to adapt to extreme conditions as well as overall genetic diversity, and increasing seed storage capacity to ensure we have seed available as we continue to conduct research to determine how best to deploy it in a changing climate (Havens et al, 2015).

Although the global momentum around climate change is building up and change is well on its way, we still need to consider how this has impacted honeybees. In efforts to survive, bees try to adapt to the rise in temperature by changing instinctive behaviors. But this doesn't take away from the fact that our crops and bees are suffering facing this adversity alone. It is stressors

like these that create a phenomenon termed as Colony Collapse Disorder (CCD). When bees have a weakened immune system and foresee death in their near future, they don't die in their hives. They fly off somewhere and die alone, which makes it a bit more challenging to study their carcasses to investigate how to remedy challenges (Amadeo, 2020). As climate change is one of the key stressors in the contribution to colony collapse disorder, it is essential to understand how the bees are responding to the changing climate change. Responses include, but are not limited to: insect larvae maturing earlier in development termed precocious foraging, and alteration of plants life cycles, causing blooming earlier in the season. Not only is there shifts in phenological events but there is also alteration in distribution (Hughes, 2000). With increasing storms tied to warming temperatures bees stay in their hives trying to wait out the storm, lasting anywhere from days to weeks. If this occurs during mating season the queen will have no choice but to lay unfertilized eggs resulting in an unwanted upward trend in male drones who serve no purpose to the hive other than mating with other queen. The unstable weather also tends to strip nectar from flowers. The conditions bees face disrupt their normal rhythm of the spring season, un-syncing pollinator activity and flowers. Continuously changing timing of life cycles between bees and flowers, caused by climate change, has the potential to disrupt a mutually beneficial relationship. The disruption could cascade throughout ecological communities, affecting frugivores, seed dispersal, and plant recruitment (Scaven and Rafferty, 2013). The prolonged droughts and elevated temperatures affect physiology of flowering plants, altering production of flowers, time of bloom, scent, height, nectar production and composition, and pollen production and composition (Scaven and Rafferty, 2013). Pollinators are also affected by warming temperature causing them to forage earlier in development, their body size at maturity and life span. The consequences *Apis mellifera* and other species endure are plentiful. Although

individually the species will not have a negative consequence to itself the physiological responses to climate could negatively impact the interactions between plant-pollinator. If the environmental stress is too much to bear, both hive bee and forager populations will decrease. This decrease has a negative impact on uncapped brood survival and forager population. To counteract mortality rate, they'll decrease the natural mortality of foragers, reducing the forager recruitment level and increasing queen's laying rate. In some cases we find wild bees like *Apis cerana cerana* activating dormant genes and signaling pathway for defense against environmental stresses.

Combined effects of nutritional stress, colony size and heat stress

To better understand the overall impact climate change has on honeybees and their productivity we consider some drivers that lead to Colony Collapse Disorder. Despite the growing concern and recognized importance of these factors for pollinator population viability, our knowledge concerning how each factor contributed to the decline of bee population is limited due to lack of controlled experimentation. The study discussed allows us to investigate each factor alone and how they influence each other. Since drivers never appear in isolated incidents in nature, we applied cross experiments to try and mimic and predict models of future effects of climate change on pollinator declines. Highlighting the important effects of heat stress and changes in bee diet under controlled and reproducible laboratory conditions, and also features striking combined negative effects of environmental stresses on colony development. Although the responses to heat stress are a bit more challenging to prescribe with certainty, we are able to discern the implication of each isolated and combined effect of each type of stress (Vanderplanck, 2019)

A bioassay was taken to evaluate the interactive effects of heat stress, a reduction of dietary resource quality and colony size. Nutrition is challenged in this because it can impact offspring number, colony size, mortality and immunity. Climate change, specifically heat stress, might increase the probability of losing favored floral resources by changing plant phenologies and distributions, thus creating mismatches between bees and their resources or by changing the quality and quantity of their floral resources. Although *Apis mellifera* is notorious for their broad range of flexibility when it comes to engaging with flowers, this should not be ruled out due to the fact it is still a confounding variable among the others. In addition, the expected increase in the frequency and intensity of extreme climate events such as heat waves can affect physiology and increase insect mortality (e.g. due to ontogenic development, changes water balance, fertility and immunity). Evolutionary adaptations to warmer conditions have occurred but only in the interiors of species ranges (Parmesan, 2006), potentially affecting the ability to detect suitable resources. Moreover, the lack of a suitable diet might decrease the resilience of organism facing heat wave in a similar manner to the stress of pesticide exposure. Pesticides are taken under consideration because they are known to alter gene expression of detoxification, behavior, immunity, and nutrition genes. After pollen feeding, the pesticide exposure alter expression of common sets of genes while also building pesticide tolerance after being consumed (Schmehl, 2014). Therefore, it is expected that any negative impact caused by heat stress will be more heightened when bees are also subjected to nutritional stress via pesticides. A closer look into how these main drivers of change affect bees is essential for understanding the nature of this decline and for the enhancement of appropriate public policies and conservation plans. With 117 colonies, Vanderplanck, (2019) conducted experiments to test the effects of three dietary quality levels under three degrees of heat stress with two colony sizes. This is done to give a spectrum of

possible outcomes. The colony size is considered to try and evaluate if sociality can buffer environmental stresses. The cross experiment was applied in effort to test the effect of (i) three distinct pollen diets displaying different amino acid concentrations and sterolic composition (i.e. low, medium and high suitability); (ii) three thermal systems (i.e. control, short and long climatic stress); and (iii) two colony sizes (i.e. small and large colonies). Bees have the ability to regulate their internal body temperature but it's limited. They have a physiological capability to regulate thoracic temperature. The thorax has heat producing muscles that are contracted to produce heat that is regulated by their ganglia this also is responsible for their flight. Blood is heated with passing through active flight muscles in the thorax and is sent near the brain. The neural output from the thoracic ganglia upon which flight control depends is highly temperature-dependent. (Heinrich, 1979). The honeybee also uses ambient external temperature to help control body temperature of individual bees. Thus, this limitation makes individuals sensitive to climate change including heat waves that have become more frequent across range in recent decades and are likely to intensify in frequency and amplitude in the future. The low inapt diets and prolonged intervals of heat stress negatively affected colony performance, and that these two effects will act interactively, with large colonies being less affected than small ones.

Experimental design for this study includes: From a total of 117 colonies, one third were nurtured at a normal temperature (26 °C, this serves as our control). The remaining colonies were divided in two groups and subjected to climatic stress (33 °C) during five (short stress) or ten (long stress) days. Colonies were fed for 28 days on diets with a dominance of *Salix* pollen (high suitability), *Cistus* pollen (medium suitability) or *Taraxacum* pollen (low suitability). Mortality, offspring production and resource collection (i.e. pollen and syrup) were monitored during or at the end of the bioassays.

Nutritional stress

Studies have shown that nutritional stresses affect pollination performance in all bee species. One of the most important variables for determining plant reproductive success is the frequency of bee visits. With behavioral changes seen i.e reduction of bee visits, impacts are seen in resource collection. This could lead to pollinator population size declining and impact pollination of wild plants and crops thus feeding into the cycle and nutritional stress being promoted.

An approach worth investigating and putting into action is to mitigate natural habitat fragmentation. Sometimes caused by natural processes like fires, floods, but mostly caused by human impacts like urbanization creating several environmental stressors that are pronounced. They include (i) chemical pollution, (ii) noise pollution, (iii) artificial night light pollution, (iv) infectious disease and (v) diet quality.(Isaksson, 2015) factors like these are known to trigger linked inflammation responses and oxidative stress which in turn affects performance via immunity. To mitigate these implications on bees is to create a landscape with wildflower strips to try and promote biodiversity and maintain pollinator networks. Our results suggest that these commercial seed mixtures should be optimized to provide a suitable diet to pollinators (i.e. generalist and specialist bees) for preserving their health and development, based on ecological constraints such as the nutritive quality of floral resources and not only on human and financial considerations (Vanderplanck, 2019)

Heat stress

Models have been developed to predict the frequency of extreme climatic events, like heat waves, and have demonstrated that by 2040 heat waves intensity and duration will increase in

North America and Europe. This will jeopardize our already at risk ecological systems. This study has found that heat stress significantly delays the developments of small colonies and low colony growth is likely due to physiological disruption to bees at several states. A phenomenon commonly seen can be attributed to heat stress, its called “Urban heat island effect,” It is described as cities being warmer compared to rural communities. This alters thermal conditions and breeding seasons by indirectly extending urban warming (Miles, 2019) .“While adult bees can tolerate a large range of temperatures, their ability to regulate brood nest temperature (which is crucial for the larval development) can be reduced when exposed to high temperatures. As the percentage of workers required for this task (e.g. via wing fanning) increases, fewer bees are available to ensure the maintenance of the colony, feeding the larvae and collect pollen and nectar. High temperatures, even for a short time, could disrupt thermoregulation and could have a negative effect on colony development which could explain the results found for small colonies (Figs 3b, 4). Bees can compensate by endothermic heat production, evaporation of water or wing fanning but such behavior incurs a substantial ergonomic cost. Such recruitment of workers for buffering high temperature could partly explain the observed decrease in resources collection in the small and large colonies bioassays (see Fig. 2a-d). In addition, despite such efforts, workers were still not able to maintain the brood temperature within the optimal range (28–32 °C). Such effect could be related to environmental stress and/or bacterial development. Overall, our data show that heat exposure represents a non-negligible risk for the survival of colonies and maintenance of pollinator populations.” (Vanderplanck, 2019). These findings highlight the importance of improving prediction of heat waves with certainty because this will help us mitigate and understand the influence of climate change on pollinators, pollination and productivity of pollinatory-depedent crops.

Colony size

The resilience between large colonies and small colonies are observed to show significant differences in coping mechanisms via social buffering abilities. The physiological effect of sociality is worth exploring especially in regard to thermoregulation as relation of thermic homeostasis of colonies within honeybees is studied. We may assume that the division of labor among foraging, brood maintenance and fanning tasks is more problematic in a small colony than in a large one with a greater number of available workers. Moreover, colonies with only some workers may not be able to maintain brood temperature when the air temperature is higher than 32 °C , compromising optimal brood development. This suggests that nutritional and/or heat stresses in early spring (i.e. when young colonies are growing and have few workers) might cause higher negative impacts than at the end of the summer (i.e. when colonies are larger with more numerous workers)” (Vanderplanck, 2019).

Combined effects

One of the more notable discoveries made in the duration of this study was the effects of exposure to heat stress seen in bees when they had access to high quality diet (*salix* diet) were significantly less intense compared to a low end diet. When high quality diets are fed to the pollinator we also see colony growth and feeding behaviors being less impacted even for small colonies considering this size was the most impacted by factors. Heat wave can cause the developmental delay of the colony and could increase the phenological mismatch between plants and pollinators. This is bad because this influences the integrity of the natural environment and puts gaps between evolutionary relationship mutualisms. “In field conditions, the synergistic

depression resulting from heat stress and diet suitability might occur during a drought-related heat wave episode with a water deficit. These events are expected to become more frequent and can decrease floral resources and/or cause a phenological drift. Consequently, the performance of bumblebee colonies (especially in arcto-alpine regions) and, bee-flower interactions could be dramatically impacted” (Vanderplanck, 2019)

In optimism, I could say we could solve or mostly delay the bees and rapidly warming world if we reduced carbon emission but in realistically there is no evidence this will be enough to mitigate the already compromised pollinators. Something more needs to be done to give the bees a chance at surviving. Therefore its essential to conserve and improve land management to minimize the impacts on bees and associated ecosystem services. A good start is to promote diversity by adding a variety of flower strips to ensure nutritional value. This is crucial especially for generalist bees like *Apis mellifera* to improve their immune systems and mitigate unfavorable pollen properties

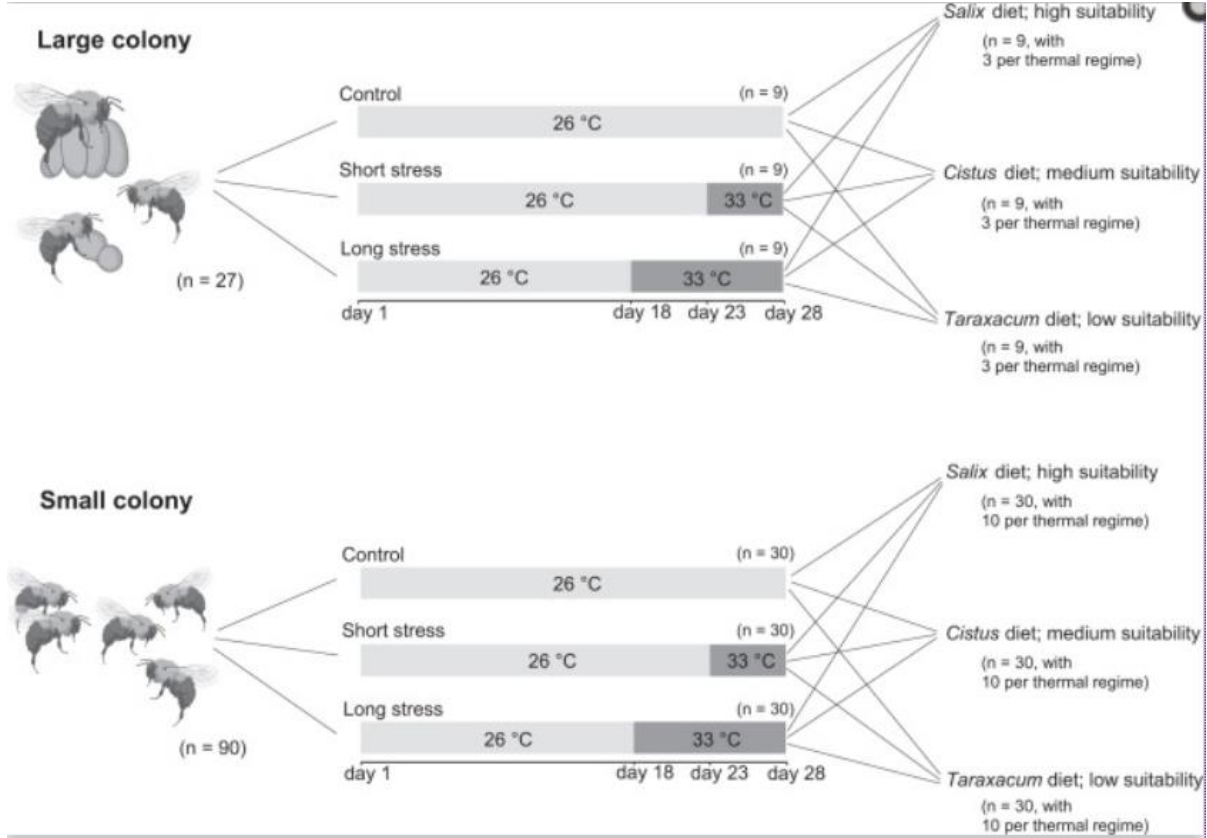
To adequately review the overall interactive effects of potential synergies between climatic and nutritional stresses we based our understanding from the performance of colonies and their feeding responses, evaluated based on colony growth, composition of brood, mortality, total pollen and syrup collection. Overall, from the data collected we deduced while both nutritional and heat stress reduced colony development there are important interactive effects that need to be addressed.

Both colony sizes are impacted by stressors, and are reflected in colony growth and mortality. The small colonies exhibited heightened reduction in male production in colonies that fed on low suitability diets (*Taraxacum*- dominant diet), indicating a slowing down in brood development (Fig.3). This is directly correlated with the decreased collection of pollen and

syrup. As duration of exposure extended to longer intervals of nutritional stress the resource collection shows that small colonies were significantly affected compared to the large colony. By reducing male production, this increased the relative importance of eggs mass, regardless of stress duration. Perhaps this has something to do with larger colonies having sociality to buffer those effects.

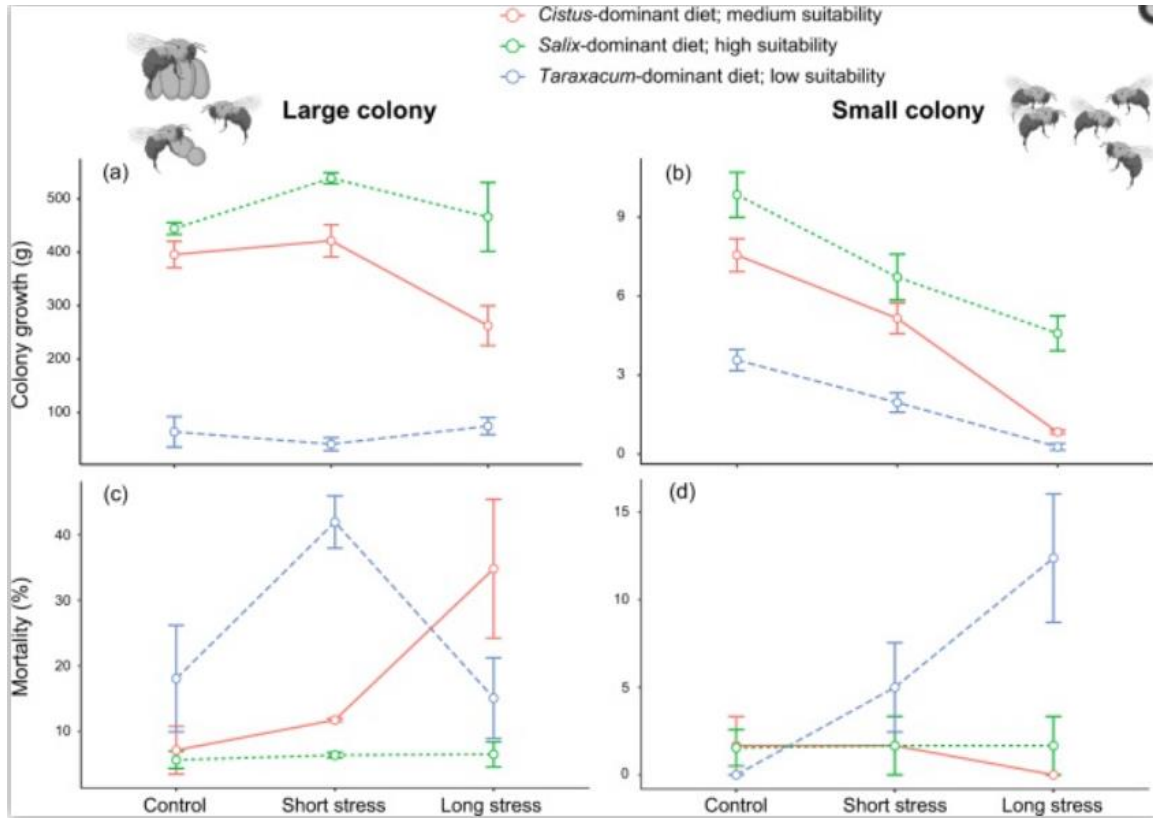
When fed a high-quality diet (*Salix*-dominant diet), these negative effects (also including mortality) are less pronounced in both small and large colonies. Rather, if fed low quality diets, mortality varied in large colonies, but increased in smaller ones. Large colonies are seen to be much more resilient. When transition in (low-medium quality diets) show an increased pollen and syrup collection, while small colonies exhibit pronounced declines in resource collection. Only when fed low sustainable diets does the large colony exhibit significant impacts compared to small colonies which are affected due to their population disadvantage.

Figure 6. Colony response to temperature stress



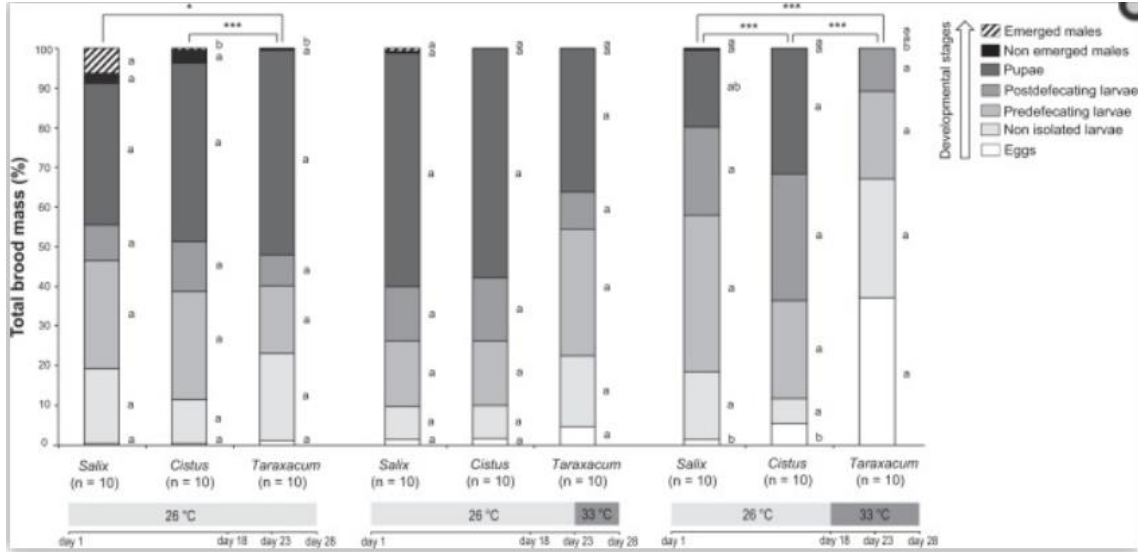
Experimental design: From a total of 117 colonies, one third were reared at a normal temperature (26 °C, control). The remaining colonies were divided in two groups, and exposed to climatic stress (33 °C) during five (short stress) or ten (long stress) days. Colonies were fed for 28 days on diets with a dominance of *Salix* pollen (high suitability), *Cistus* pollen (medium suitability) or *Taraxacum* pollen (low suitability). Mortality, offspring production and resource collection (i.e. pollen and syrup) were monitored during or at the end of the bioassays. (Vanderplanck, 2019)

Figure 7. Colony development.



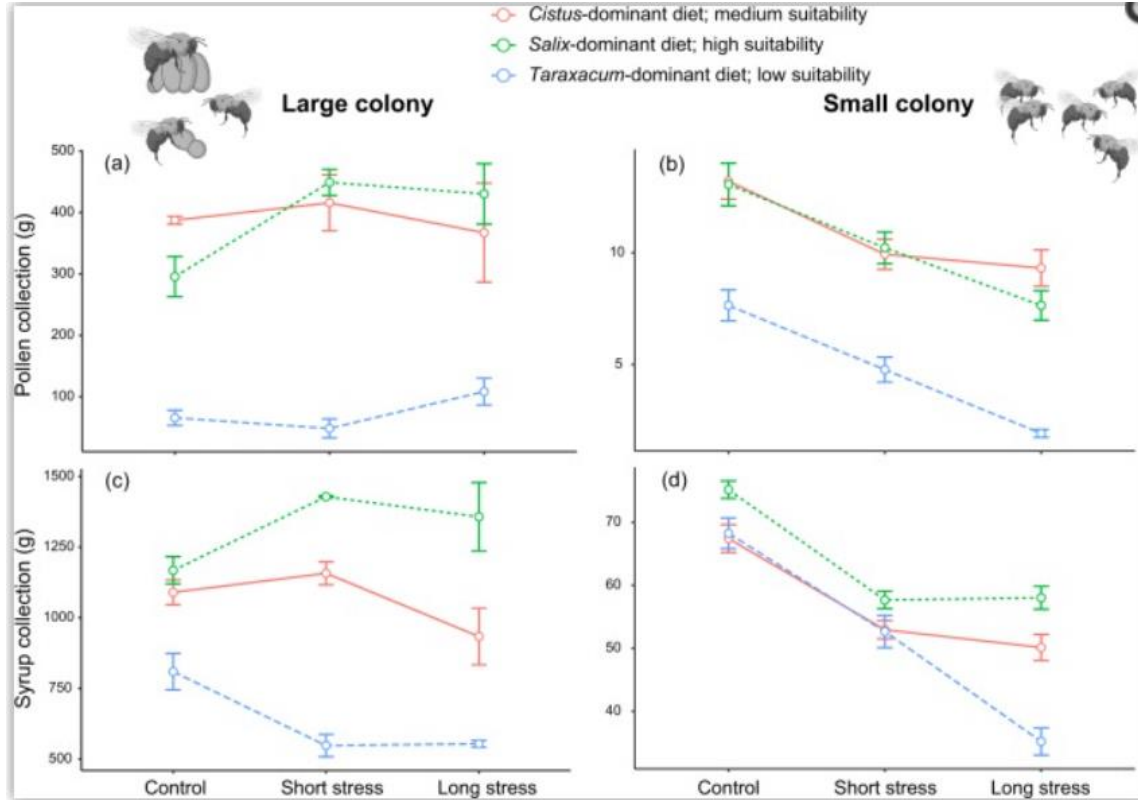
Colony growth (a,b) and mortality (c,d) for large (left) and small (right) colonies exposed to different levels of environmental stresses (mean \pm SE). Diet with a dominance of *Salix* sp. is highly suitable, diet with a dominance of *Cistus* sp. has medium suitability, and diet with a dominance of *Taraxacum* sp. has low suitability. (Vanderplanck, 2019)

Figure 8. Small colony dynamics



Brood composition at different developmental stages expressed as percentage of total brood mass (i.e. dynamics of micro-colony development) for small colonies exposed to different levels of environmental stresses. Diet with a dominance of *Salix* sp. is highly suitable, diet with a dominance of *Cistus* sp. has medium suitability, and diet with a dominance of *Taraxacum* sp. has low suitability. Asterisks indicate significant differences in brood composition between micro-colonies fed different pollen diets (pairwise perMANOVAs; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Different letters indicate significant differences in the proportion of brood stages among bioassays (post-hoc tests, $p < 0.05$). (Vanderplanck, 2019)

Figure 9. Resource collection by large and small colonies.



Pollen collection (**a,b**) and syrup collection (**c,d**) for large (left) and small (right) colonies exposed to different levels of environmental stresses (mean \pm SE). Diet with a dominance of *Salix* sp. is highly suitable, diet with a dominance of *Cistus* sp. has medium suitability, and diet with a dominance of *Taraxacum* sp. has low suitability. (Vanderplanck, 2019).

CHAPTER IV

PROMISING SOLUTIONS FOR COLONY RECOVERY

Joe Rogan had guest Paul Stamets on his podcast where he talked about how fungi can super jump bee's immunity doubling their lifespan while also reducing susceptibility to viruses and bacterial infections. About 12,000 years ago, we founded agriculture which led us to deforestation.

When we began cutting down trees we started breaking down the immunological network of nature, specifically mycelium. The fact that these same mushroom reduced viruses in bees, pigs, birds, people, speaks to a bigger concept; that mycelium is a part of the ecosystem's immunity. As we lose the debris fields that mycelium are dependent upon we began to dismantle the immunological health of our environment and zoonotic diseases coming from factory farms increases. Mushroom called *Agarikon* and grows exclusively in the old-growth forests of the Pacific Northwest, is believed to be the longest living mushroom in north America. It's a perennial polypore, it looks like a giant beehive by coincidence up on a tree. The *Agarikon* was a type of mushroom that are duly active against bacteria and viruses, this is medically significant and a great advocate for natural products because you have a consortium of protective agents that reside in their extract. Due to the increase in monocultured landscapes and loss of biodiversity, bees have lost access to many sources of nutrition that they might have benefited from in the past. Mycelium extracts may prove to be a powerful support for bees as they endure more challenging conditions in our ecosystems.

If we pay attention to the vast resources bionetworks have to offer within in the biodiversity of the ecosystems that are still intact, we potentially have an untapped revenue to vaccines or adjuvants that help upregulate immunity. Stamets argues that we need to save and

preserve the old growth forest as a matter of national defense, inhabiting fungal genomes are essential to our future and present survival. The more we eradicate these landscapes of biodiversity the more we lose potential agents that can fight off disease. Paul Stamets partnered with Washington State University to protect honeybees and pollinators. Their global research program collaborates with apiculturists, scientists, environmentalists and communities to improve honey bee and pollinator health in effort advocate research on how fungi can help honey bees. Paul Stamets and Paul Taylor actually designed a delivery system for mushroom mycelium extract that anyone can participate in to aid health and nutrition for bees all across landscapes, its called BeeMushroomed Feeder. The patent is still pending and is currently still in development, but it will groundbreaking.

Over at Washington State University they conduct research geared towards population genetics and systemic research on honeybees and other organisms. They are especially interested in genetic changes tied to honeybee colonization events, pest introduction and host shifts of phytophagous insects. ("WSU HONEY BEES + POLLINATORS | Washington State University") Field research in the apiaries should be centered on projects addressing problems of major importance to beekeepers. With 60 different potential stressors targeting honeybees if we set aside funding for studies for the improvement of apiaries, apiculturist and all surrounding impacts we could find solutions at a exponential rate. If beekeepers were more knowledgeable of their bee problems and the solutions they choose to implicate we could collect raw data to quantitatively study what solutions aid best and potentially be able to start rehabilitation programs to help mitigate and achieve bee wealth.

According to Klein et al., (2007), a way to effectively redirect our future efforts is to focus on increasing biodiversity to stabilize our ecosystem because "the stability of crop yields

not only depends on pollination, but also on further ecosystem services," Therefore, we need to manage landscapes carefully for a diversity of functionally important groups of organisms that sustain important ecosystem services such as pollination, pest, pathogen and weed control, and decomposition." (Kremen, 2006). Conservation approaches that integrate biodiversity will promote natural adaption into land use and are complementary. As we see bees facing a variety of stresses each waring down their already distressed immune systems causing CCD, then the complementary system that is put into place should restore natural ecological network and resilience. (Schmitz, 2015)

CHAPTER V

SUMMARY AND CONCLUSION

Many stressors contribute to the decline of honeybees one being Colony Collapse Disorder. Scientist have a hard time prescribing what exactly induces this phenomenon. This disorder happens so suddenly, a hive can be flourishing and just 2 weeks later majority of bees abandon hive and leave a few nurse bees to attend to the brood and the queen with pounds of honey. The lack of carcasses to study result in no indicator of what led to their departure. There are many theories that contribute to their demise but are unable to support with certainty. These stressors are both biotic and abiotic and the lack of biodiversity stunts pollinators immune system, constantly overwhelmed with pesticides, urbanization, climate change, and many more they are unable to persist with comfort. When faced with adversity society in large colonies acts as a buffer increasing their resilience against disadvantageous factors. Paul Stamets had the right idea about using fungi to nurture bees back to health. Nature has provided us with countless treatments for diseases that we were unable to develop synthetically that was as effective. At times, unfortunately we are only able to treat symptoms and not the source of the problem whether it be a virus or any other predator. Nature allowed us to derive aspirin from *Aspirina* (*Salix alba*) and Morphine from *Morfina* (*Papaver somniferum*) and now we are able to gather extracts from a mushroom that will literally double the lifespan of a honeybee and give it its ability to fight off viruses and bacterial infections. Nature will find a way to support ecosystems if we look close enough. For instance, when a bear scratches a tree with its claw resin comes out; bees are attracted to the propolis or more so its raw ingredient—resin. It collects resin and they use it to reinforce their hive from viruses and bacteria. Resin is made by plants to protect it from

predators, so when it is damaged, the resin oozes out of cells and covers the injury and harden when exposed to air, much like our scabs. Bees are also attracted to rotted wood that promote the growth of mycelium because of the immunological benefits. Deforestation is more prevalent than ever thus mycelium doesn't have a habitat and is unable decompose wood and produce the compounds bees are attracted too which renders the bees immunologically deficient in another aspect.

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